

WO0120037

Publication Title:

PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR ALPHA AND
DISORDERS OF LIPID METABOLISM

Abstract:

Abstract of WO0120037

The complete genomic structure of the peroxisome proliferator-activated receptor alpha gene is described. Also described are the identification of novel single nucleotide polymorphisms and association with higher plasma LDL-cholesterol and total and LDL-apolipoprotein B concentrations. Data supplied from the esp@cenet database - Worldwide

Courtesy of <http://v3.espacenet.com>

BEST AVAILABLE COPY

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
22 March 2001 (22.03.2001)

PCT

(10) International Publication Number
WO 01/20037 A2

(51) International Patent Classification⁷: C12Q 1/68,
C07K 14/47, A61K 38/00

(21) International Application Number: PCT/US00/25124

(22) International Filing Date:
14 September 2000 (14.09.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/154,736 17 September 1999 (17.09.1999) US

(71) Applicants (for all designated States except US):
COMPLEXE HOSPITALIER DE LA SAGAMIE
[CA/CA]; Unité de Recherche Clinique, 305, rue St
Vallier, Chicoutimi, Quebec G7H 7H6 (CA). MCGILL
UNIVERSITY [CA/CA]; 845 Sherbrooke Street West,
Montreal, Quebec H3A 2T5 (CA).

(72) Inventors; and

(75) Inventors/Applicants (for US only): HUDSON, Thomas,
J. [CA/CA]; 631 Metcalfe Avenue, Westmount, Quebec

H3Z 2J2 (CA). GAUDET, Daniel [CA/CA]; 315 Cha-
banel Street, Chicoutimi, Quebec G7H 3S1 (CA). VOHL,
Marie-Claude [CA/CA]; 1018 Jean Charles Cantin,
Cap-Rouge, Quebec G1Y 2X1 (CA). BREWER, Carl
[US/CA]; 5259 Borden Avenue, Montreal, Quebec H4V
2T2 (CA). MORGAN, Kenneth [US/CA]; 1745 Cedar
Avenue, Apt# 809, Montreal, Quebec H3G 1A7 (CA).

(74) Agents: HOGLE, Doreen, M. et al.; Hamilton, Brook,
Smith & Reynolds, P.C., Two Militia Drive, Lexington, MA
02421 (US).

(81) Designated States (national): AU, CA, JP, MX, US.

(84) Designated States (regional): European patent (AT, BE,
CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC,
NL, PT, SE).

Published:

— Without international search report and to be republished
upon receipt of that report.

For two-letter codes and other abbreviations, refer to the "Guid-
ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.



WO 01/20037 A2

(54) Title: PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR ALPHA AND DISORDERS OF LIPID METABOLISM

(57) Abstract: The complete genomic structure of the peroxisome proliferator-activated receptor alpha gene is described. Also described are the identification of novel single nucleotide polymorphisms and association with higher plasma LDL-cholesterol and total and LDL-apolipoprotein B concentrations.

PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR ALPHA
AND DISORDERS OF LIPID METABOLISM

RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional application Serial No.
5 60/154,736, filed September 17, 1999, the entire teachings of which are incorporated
herein by reference.

BACKGROUND OF THE INVENTION

Peroxisome proliferator-activated receptors (PPARs) are members of a large
family of ligand-inducible transcription factors that includes receptor for retinoids
10 and vitamin D as well as thyroid and steroid hormones (1). PPARs regulate the
expression of target genes by binding to DNA sequence elements, termed PPAR
response elements (PPRE) (2,3). PPREs have been identified in the regulatory
regions of a variety of genes involved in lipid and energy balance. Three different
PPAR genes have been identified (α , δ and γ) (2,3). PPAR γ , expressed mainly in
15 adipose tissue, regulates adipocyte differentiation (4,5). PPAR α , the first to be
identified, is expressed mainly in tissues that have a high level of fatty acid (FA)
catabolism such as the liver, kidney, heart and muscle (6,7). Numerous studies have
demonstrated that several genes encoding enzymes involved in β and ω -oxidation
are under the control of PPAR α (2,3). PPAR α is activated by medium and very
20 long chain FA and polyunsaturated FA, such as eicosapentaenoic acid (8). In
addition to its role in FA oxidation, PPAR α appears to play an important role in the
control of extracellular lipid metabolism. In fact, a variety of hypolipidemic fibrates
are synthetic ligands for PPAR α (9).

FAs are essential biological components and are used as metabolic fuels,
25 covalent regulators of signaling molecules, and essential components of cellular
membranes. Thus, FA must be kept within narrow physiological limits. Altered
levels of FA are linked to a variety of metabolic diseases including atherosclerosis,
hyperlipidemia, obesity, insulin resistance and type 2 diabetes (10, 11).

SUMMARY OF THE INVENTION

Peroxisome proliferator-activated receptor alpha (PPAR α) is a member of the steroid hormone receptor super family that is involved in the control of cellular lipid utilization. As described herein, the genomic structure of the human PPAR α was determined, and intronic primers were designed to sequence the complete coding region and the exon-intron boundaries of the human PPAR α gene among 12 patients with type 2 diabetes and 2 controls. Sequence analyses revealed the presence of a L162V missense mutation in exon 5; that is, there is a C to G transversion in the first position of codon 162 leading to a substitution of a valine for the leucine at that position. Mutation L162V is contained within the DNA binding domain of the human PPAR α gene, and leucine 162 is highly conserved among humans, mice, rats and guinea pigs. Among diabetics and controls, a trend was observed toward higher plasma LDL-cholesterol and apolipoprotein B levels among carriers of the rare V162 allele. To verify these findings, an independent cohort of 193 non-diabetic subjects recruited in the greater Quebec City area was screened. Comparison of the lipoprotein-lipid profile between L162 homozygotes and carriers of the rare V162 allele showed significant differences in plasma LDL-cholesterol, total and LDL-apolipoprotein B concentrations, carriers of the V162 allele having the highest levels. These results suggest that the rare V162 allele may make the subjects more prone to develop a hyperapobetalipoproteinemia.

Thus, the invention relates to the SNPs identified as described herein, both singly and in combination, as well as to the use of these SNPs, and others in PPAR (e.g., PPAR α) genes, particularly those nearby in linkage disequilibrium with these SNPs, for diagnosis, prediction of clinical course and treatment response for disorders of lipid metabolism, development of new treatments for disorders of lipid metabolism based upon comparison of the variant and normal versions of the gene or gene product, and development of cell-culture based and animal models for research and treatment of disorders of lipid metabolism. The invention also relates to methods for diagnosing and treating disorders of lipid metabolism, especially high LDL cholesterol levels and hyperapobetalipoproteinemias, and to methods for identifying compounds for use in the diagnosis and treatment of said disorders. The

-3-

invention further relates to novel compounds and pharmaceutical compositions for use in the diagnosis and treatment of disorders of lipid metabolism.

The invention relates to isolated nucleic acid molecules comprising all or a portion of the variant allele of PPAR α (e.g., wherein reference or wildtype PPAR α is exemplified by SEQ ID NO: 1). Preferred portions are at least 10 contiguous nucleotides and comprise the polymorphic site, e.g., a portion of SEQ ID NO: 1 which is at least 10 contiguous nucleotides and comprises the "G" at the first position of codon 162 (nucleotide 696) of the PPAR α gene, or a portion of SEQ ID NO: 1 which is at least 10 contiguous nucleotides and comprises the "A" at the last position of codon 253 (nucleotide 971) of the PPAR α gene. The invention further relates to isolated gene products, e.g., polypeptides or proteins, which are encoded by a nucleic acid molecule comprising all or a portion of the variant allele of PPAR α .

The invention further relates to isolated proteins or polypeptides comprising all or a portion of the variant amino acid sequence of PPAR α (e.g., wherein reference or wildtype PPAR α is exemplified by SEQ ID NO: 2), and to isolated proteins or polypeptides comprising all or a portion of the variant amino acid sequence of PPAR α . Preferred polypeptides are at least 10 contiguous amino acids and comprise the polymorphic amino acid, e.g., a portion of SEQ ID NO: 2 which is at least 10 contiguous amino acids and comprises the valine at residue 162. The invention further relates to isolated nucleic acid molecules encoding such proteins and polypeptides, as well as to antibodies which bind, e.g., specifically, to such proteins and polypeptides.

In one embodiment, the invention relates to a method for predicting the likelihood that an individual will have a disorder of lipid metabolism (or diagnosing or aiding in the diagnosis of a disorder of lipid metabolism), e.g., high LDL cholesterol levels, atherosclerosis, coronary heart disease and/or hyperapobetalipoproteinemias, comprising the steps of obtaining a DNA sample from an individual to be assessed and determining the nucleotide present at the first position of codon 162 (nucleotide 696) of the PPAR α gene. The presence of a "C" (the reference nucleotide) at this position indicates that the individual has a lower likelihood of having a disorder of lipid metabolism than an individual having a "G"

at that position, or a greater likelihood of having less severe symptomology. In a preferred embodiment, the disorder is high LDL cholesterol levels, atherosclerosis and/or coronary heart disease. In another embodiment, the disorder is a hyperapobetalipoproteinemia. In a particular embodiment, the individual is an individual at risk for development of a disorder of lipid metabolism.

In another embodiment, the invention relates to a method for predicting the likelihood that an individual will have a disorder of lipid metabolism (or diagnosing or aiding in the diagnosis of a disorder of lipid metabolism), e.g., high LDL cholesterol levels, atherosclerosis, coronary heart disease and/or hyperapobetalipoproteinemias, comprising the steps of obtaining a DNA sample from an individual to be assessed and determining the nucleotide present at the first position of codon 162 (nucleotide 696) of the PPAR α gene. The presence of a "G" (the variant nucleotide) at this position indicates that the individual has a greater likelihood of having a disorder of lipid metabolism than an individual having a "C" at that position, or a greater likelihood of having more severe symptomology. In a preferred embodiment, the disorder is high LDL cholesterol levels, atherosclerosis and/or coronary heart disease. In another embodiment, the disorder is a hyperapobetalipoproteinemia. In a particular embodiment, the individual is an individual at risk for development of a disorder of lipid metabolism.

In another embodiment, the invention relates to a method for predicting the likelihood that an individual will have a disorder associated with a "G" at nucleotide position 696 of the PPAR α gene (or diagnosing or aiding in the diagnosis of such a disorder) comprising the steps of obtaining a DNA sample from an individual to be assessed and determining the nucleotide present at position 696 of the PPAR α gene. The presence of a "G" at this position indicates that the individual has a greater likelihood of having a disorder associated therewith than an individual having a "C" at that position, or a greater likelihood of having more severe symptomology. Conversely, the presence of a "C" at this position indicates that the individual has a lower likelihood of having a disorder associated therewith than an individual having a "G" at that position, or a greater likelihood of having less severe symptomology. In a preferred embodiment, the disorder is high LDL cholesterol levels, atherosclerosis and/or coronary heart disease. In another embodiment, the disorder is a

hyperapobetalipoproteinemia. In a particular embodiment, the individual is an individual at risk for development of such a disorder.

In another embodiment, the invention relates to a method for predicting the likelihood that an individual will have a disorder associated with a "C" at nucleotide
5 position 696 of the PPAR α gene (or diagnosing or aiding in the diagnosis of such a disorder) comprising the steps of obtaining a DNA sample from an individual to be assessed and determining the nucleotide present at position 696 of the PPAR α gene. The presence of a "C" at this position indicates that the individual has a greater likelihood of having a disorder associated therewith than an individual having a "G"
10 at that position, or a greater likelihood of having more severe symptomology. Conversely, the presence of a "G" at this position indicates that the individual has a lower likelihood of having a disorder associated therewith than an individual having a "G" at that position, or a greater likelihood of having less severe symptomology. In a preferred embodiment, the disorder is high LDL cholesterol levels,
15 atherosclerosis and/or coronary heart disease. In another embodiment, the disorder is a hyperapobetalipoproteinemia. In a particular embodiment, the individual is an individual at risk for development of such a disorder.

In another embodiment, the invention relates to a method for predicting the likelihood that an individual will have a disorder associated with an "A" at
20 nucleotide position 971 of the PPAR α gene (or diagnosing or aiding in the diagnosis of such a disorder) comprising the steps of obtaining a DNA sample from an individual to be assessed and determining the nucleotide present at position 971 of the PPAR α gene. The presence of an "A" at this position indicates that the individual has a greater likelihood of having a disorder associated therewith than an
25 individual having a "G" at that position, or a greater likelihood of having more severe symptomology. Conversely, the presence of a "G" at this position indicates that the individual has a lower likelihood of having a disorder associated therewith than an individual having a "A" at that position, or a greater likelihood of having less severe symptomology. In a particular embodiment, the individual is an
30 individual at risk for development of such a disorder.

In another embodiment, the invention relates to a method for predicting the likelihood that an individual will have a disorder associated with a "G" at nucleotide

position 971 of the PPAR α gene (or diagnosing or aiding in the diagnosis of such a disorder) comprising the steps of obtaining a DNA sample from an individual to be assessed and determining the nucleotide present at position 971 of the PPAR α gene. The presence of a "G" at this position indicates that the individual has a greater
5 likelihood of having a disorder associated therewith than an individual having a "A" at that position, or a greater likelihood of having more severe symptomology. Conversely, the presence of an "A" at this position indicates that the individual has a lower likelihood of having a disorder associated therewith than an individual having a "G" at that position, or a greater likelihood of having less severe symptomology.

10 In a particular embodiment, the individual is an individual at risk for development of such a disorder.

In one embodiment, the invention relates to a method for predicting the likelihood that an individual will have a disorder of lipid metabolism (or diagnosing or aiding in the diagnosis of a disorder of lipid metabolism), e.g., high LDL
15 cholesterol levels, atherosclerosis, coronary heart disease and/or hyperapobetalipoproteinemias, comprising the steps of obtaining a biological sample comprising the PPAR α protein or relevant portion thereof from an individual to be assessed and determining the amino acid present at amino acid position 162 of the PPAR α protein. The presence of a leucine (the reference amino acid) at this
20 position indicates that the individual has a lower likelihood of having a disorder of lipid metabolism than an individual having a valine at that position, or a greater likelihood of having less severe symptomology. In a preferred embodiment, the disorder is high LDL cholesterol levels, atherosclerosis and/or coronary heart disease. In another embodiment, the disorder is a hyperapobetalipoproteinemia. In
25 a particular embodiment, the individual is an individual at risk for development of a disorder of lipid metabolism.

In another embodiment, the invention relates to a method for predicting the likelihood that an individual will have a disorder of lipid metabolism (or diagnosing or aiding in the diagnosis of a disorder of lipid metabolism), e.g., high LDL
30 cholesterol levels, atherosclerosis, coronary heart disease and/or hyperapobetalipoproteinemias, comprising the steps of obtaining a biological sample comprising the PPAR α protein or relevant portion thereof from an individual to be

assessed and determining the amino acid present at amino acid position 162 of the PPAR α protein. The presence of a valine (the variant amino acid) at this position indicates that the individual has a greater likelihood of having a disorder of lipid metabolism than an individual having a leucine at that position, or a greater
5 likelihood of having more severe symptomology. In a preferred embodiment, the disorder is high LDL cholesterol levels, atherosclerosis and/or coronary heart disease. In another embodiment, the disorder is a hyperapobetalipoproteinemia. In a particular embodiment, the individual is an individual at risk for development of a disorder of lipid metabolism.

10 In another embodiment, the invention relates to a method for predicting the likelihood that an individual will have a disorder associated with a valine at amino acid position 162 of the PPAR α protein (or diagnosing or aiding in the diagnosis of such a disorder) comprising the steps of obtaining a biological sample comprising the PPAR α protein or relevant portion thereof from an individual to be assessed and
15 determining the amino acid present at amino acid position 162 of the PPAR α protein. The presence of a valine at this position indicates that the individual has a greater likelihood of having a disorder associated therewith than an individual having a leucine at that position, or a greater likelihood of having more severe symptomology. Conversely, the presence of a leucine at this position indicates that
20 the individual has a lower likelihood of having a disorder associated therewith than an individual having a valine at that position, or a greater likelihood of having less severe symptomology. In a preferred embodiment, the disorder is high LDL cholesterol levels, atherosclerosis and/or coronary heart disease. In another embodiment, the disorder is a hyperapobetalipoproteinemia. In a particular
25 embodiment, the individual is an individual at risk for development of such a disorder.

In another embodiment, the invention relates to a method for predicting the likelihood that an individual will have a disorder associated with a leucine at amino acid position 162 of the PPAR α protein (or diagnosing or aiding in the diagnosis of
30 such a disorder) comprising the steps of obtaining a biological sample comprising the PPAR α protein or relevant portion thereof from an individual to be assessed and determining the amino acid present at amino acid position 162 of the PPAR α

protein. The presence of a leucine at this position indicates that the individual has a greater likelihood of having a disorder associated therewith than an individual having a valine at that position, or a greater likelihood of having more severe symptomology. Conversely, the presence of a valine at this position indicates that the individual has a lower likelihood of having a disorder associated therewith than an individual having a leucine at that position, or a greater likelihood of having less severe symptomology. In a preferred embodiment, the disorder is high LDL cholesterol levels, atherosclerosis and/or coronary heart disease. In another embodiment, the disorder is a hyperapobetalipoproteinemia. In a particular embodiment, the individual is an individual at risk for development of such a disorder.

In another embodiment, the invention relates to pharmaceutical compositions comprising a reference or variant PPAR α gene or gene product, or biologically active portion thereof. The invention relates to pharmaceutical compositions comprising a reference PPAR α gene or gene product, or biologically active portion thereof, for use in the treatment of lipid metabolism disorders. The invention further relates to the use of compositions (i.e., agonists and antagonists) which enhance or increase or which reduce or decrease, respectively, the activity of a PPAR α gene product for use in the treatment of disorders of lipid metabolism.

BRIEF DESCRIPTION OF THE DRAWING

Figure 1 shows a schematic representation of the human PPAR α gene. The upper portion is a representation of the human PPAR α messenger RNA. The coding region is shaded and arrows indicate initiator and stop codons. The spatial localization of exons within the gene is shown in the lower portion. Intron sizes are also indicated. The gene spans 83.7 Kb of genomic DNA.

Figure 2 shows a nucleotide sequence (SEQ ID NO: 1) of the PPAR α mRNA.

Figure 3 shows an amino acid sequence (SEQ ID NO: 2) of the PPAR α protein.

DETAILED DESCRIPTION OF THE INVENTION

A list of references referred to herein by number can be found at the end of the Specification.

The human PPAR α gene has been mapped on chromosome 22 slightly
5 telomeric to a linkage group of six genes and genetic markers located in the region
22q12-q13.1 (24). As described herein, the structure of the human PPAR α gene has
been determined. This will facilitate genetics studies evaluating the role of this gene
in insulin resistance, type 2 diabetes and dyslipidemia. The human gene spans about
83.7 Kb and is composed of 8 exons. The 5' donor and 3' acceptor splice sites were
10 found to be in agreement to the consensus splice donor and acceptor sequences. The
position of introns separating coding exons is conserved between the human and
mouse genes (25). However, the sequence of the non-coding portion of the gene
shows no significant homology across the two species (24,25). It has been observed
that the expression of PPAR α mRNA in liver is significantly lower in humans
15 (26,27).

As described herein, direct sequencing of the entire coding region of the
human PPAR α gene as well as exon-intron boundaries revealed the presence of a
silent G to A substitution in exon 7 (nucleotide 971 of the PPAR α gene). This
substitution does not contribute to an amino acid change, as both polymorphic forms
20 encode a threonine at amino acid position 253, but is considered part of the subject
application; for example, this polymorphism can be used to genotype PPAR α or in a
diagnostic method for disorders associated with one or the other allele. Sequence
analyses also revealed the presence of a L162V missense mutation (polymorphism)
in exon 5; that is, there is a C to G transversion in the first position of codon 162
25 (nucleotide 696), leading to a substitution of a valine for a leucine at amino acid 162.
In this instance, "C" is considered the reference nucleotide and "G" is considered the
variant nucleotide.

As used herein, polymorphism refers to the occurrence of two or more
genetically determined alternative sequences or alleles in a population. A
30 polymorphic marker or site is the locus at which divergence occurs. Preferred
markers have at least two alleles, each occurring at frequency of greater than 1%,
and more preferably greater than 10% or 20% of a selected population. A

polymorphic locus may be as small as one base pair, in which case it is referred to as a single nucleotide polymorphism.

The contribution of the L162V missense mutation to the modulation of the lipoprotein-lipid profile was examined. A trend toward higher LDL-cholesterol and plasma total-apolipoprotein B levels was seen among carriers of the V162 allele of the diabetes case-control cohort. The use of a Quebec City non-diabetic male cohort confirmed this tendency, as carriers of the V162 allele had the highest LDL-cholesterol, total and LDL-apolipoprotein B levels. The presence of the V162 allele was associated with a 10.6, 10.3 and 11.7% increase in plasma LDL-cholesterol, total and LDL-apolipoprotein B levels, respectively. These results suggest that the PPAR α L162V missense mutation may modulate the concentration of apolipoprotein B-containing lipoproteins. Thus, carriers of the rare V162 allele may be more prone to develop atherosclerosis and subsequent coronary heart disease, since increased levels of LDL-cholesterol and apolipoprotein B levels are well known risk factors for heart disease (28-31).

Thus, it is possible that changes in PPAR α activity could contribute to the observed lipoprotein-lipid variability in the population. Results presented herein suggest that the L162V mutation may modulate plasma lipoprotein-lipid levels. This missense mutation results in a non-conservative amino acid substitution located within the DNA binding domain or C domain of the protein (26). This domain, which targets the receptor to specific DNA sequences known as hormone response element (HRE) or PPRE for PPAR specific genes, is the most conserved among nuclear receptor domains, (2,3,26). Taken together, these observations suggest that the leucine 162 may be important for the activity of PPAR α and thus the valine substitution may alter its activity. It is also possible that the L162V mutation is in linkage disequilibrium with a functional mutation in another gene in the neighborhood that regulates blood lipid concentrations.

The invention relates to isolated nucleic acid molecules comprising all or a portion of the variant allele of PPAR α (e.g., wherein reference or wildtype PPAR α is exemplified by SEQ ID NO: 1). Preferred portions are at least 10 contiguous nucleotides and comprise the polymorphic site, e.g., a portion of SEQ ID NO: 1 which is at least 10 contiguous nucleotides and comprises the "G" at the first

position of codon 162 (nucleotide 696) of the PPAR α gene, or a portion of SEQ ID NO: 1 which is at least 10 contiguous nucleotides and comprises the "A" at the last position of codon 253 (nucleotide 971) of the PPAR α gene. The invention further relates to isolated gene products, e.g., polypeptides or proteins, which are encoded
5 by a nucleic acid molecule comprising all or a portion of the variant allele of PPAR α .

A nucleic acid molecule or oligonucleotide can be DNA or RNA, and single- or double-stranded. Nucleic acid molecules and oligonucleotides can be naturally occurring or synthetic, but are typically prepared by synthetic means. Preferred
10 nucleic acid molecules and oligonucleotides of the invention include segments of DNA, or their complements, which include any one of the polymorphic sites shown in the Table. The segments can be between 5 and 250 bases, and, in specific embodiments, are between 5-10, 5-20, 10-20, 10-50, 20-50 or 10-100 bases. For example, the segment can be 21 bases. The polymorphic site can occur within any
15 position of the segment.

The invention also relates to nucleic acid molecules which hybridize to the variant alleles identified herein (or their complements) and which also comprise the variant nucleotide at the SNP site. Hybridizations are usually performed under stringent conditions, for example, at a salt concentration of no more than 1 M and a
20 temperature of at least 25°C. For example, conditions of 5X SSPE (750 mM NaCl, 50 mM NaPhosphate, 5 mM EDTA, pH 7.4) and a temperature of 25-30°C, or equivalent conditions, are suitable for allele-specific probe hybridizations. Equivalent conditions can be determined by varying one or more of the parameters given as an example, as known in the art, while maintaining a similar degree of
25 identity or similarity between the target nucleotide sequence and the primer or probe used.

The invention also relates to nucleic acid molecules which share substantial sequence identity to the variant alleles identified herein (or their complements) and which also comprise the variant nucleotide at the SNP site. Particularly preferred
30 are nucleic acid molecules and fragments which have at least about 60%, preferably at least about 70, 80 or 85%, more preferably at least about 90%, even more preferably at least about 95%, and most preferably at least about 98% identity with

nucleic acid molecules described herein. The percent identity of two nucleotide or amino acid sequences can be determined by aligning the sequences for optimal comparison purposes (*e.g.*, gaps can be introduced in the sequence of a first sequence). The nucleotides or amino acids at corresponding positions are then compared, and the percent identity between the two sequences is a function of the number of identical positions shared by the sequences (*i.e.*, % identity = # of identical positions/total # of positions x 100). In certain embodiments, the length of a sequence aligned for comparison purposes is at least 30%, preferably at least 40%, more preferably at least 60%, and even more preferably at least 70%, 80% or 90% of the length of the reference sequence. The actual comparison of the two sequences can be accomplished by well-known methods, for example, using a mathematical algorithm. A preferred, non-limiting example of such a mathematical algorithm is described in Karlin *et al.*, *Proc. Natl. Acad. Sci. USA*, 90:5873-5877 (1993). Such an algorithm is incorporated into the NBLAST and XBLAST programs (version 2.0) as described in Altschul *et al.*, *Nucleic Acids Res.*, 25:389-3402 (1997). When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (*e.g.*, NBLAST) can be used. See <http://www.ncbi.nlm.nih.gov>. In one embodiment, parameters for sequence comparison can be set at score=100, wordlength=12, or can be varied (*e.g.*, W=5 or W=20).

The term "isolated" is used herein to indicate that the material in question exists in a physical milieu distinct from that in which it occurs in nature. For example, an isolated nucleic acid of the invention may be substantially isolated with respect to the complex cellular milieu in which it naturally occurs. In some instances, the isolated material will form part of a composition (for example, a crude extract containing other substances), buffer system or reagent mix. In other circumstance, the material may be purified to essential homogeneity, for example as determined by PAGE or column chromatography such as HPLC. Preferably, an isolated nucleic acid comprises at least about 50, 80 or 90 percent (on a molar basis) of all macromolecular species present.

Thus, the invention relates to a method for predicting the likelihood that an individual will have a disorder of lipid metabolism (or diagnosing or aiding in the

diagnosis of a disorder of lipid metabolism), e.g., high LDL cholesterol levels, atherosclerosis, coronary heart disease and/or hyperapobetalipoproteinemias, comprising the steps of obtaining a DNA sample from an individual to be assessed and determining the nucleotide present at the first position of codon 162 (nucleotide
5 696) of the PPAR α gene. The presence of a "C" (the reference nucleotide) at this position indicates that the individual has a lower likelihood of having a disorder of lipid metabolism than an individual having a "G" at that position, or a greater likelihood of having less severe symptomology. In a preferred embodiment, the disorder is high LDL cholesterol levels, atherosclerosis and/or coronary heart
10 disease. In another embodiment, the disorder is a hyperapobetalipoproteinemia. In a particular embodiment, the individual is an individual at risk for development of a disorder of lipid metabolism.

In another embodiment, the invention relates to a method for predicting the likelihood that an individual will have a disorder of lipid metabolism (or diagnosing
15 or aiding in the diagnosis of a disorder of lipid metabolism), e.g., high LDL cholesterol levels, atherosclerosis, coronary heart disease and/or hyperapobetalipoproteinemias, comprising the steps of obtaining a DNA sample from an individual to be assessed and determining the nucleotide present at the first position of codon 162 (nucleotide 696) of the PPAR α gene. The presence of a "G"
20 (the variant nucleotide) at this position indicates that the individual has a greater likelihood of having a disorder of lipid metabolism than an individual having a "C" at that position, or a greater likelihood of having more severe symptomology. In a preferred embodiment, the disorder is high LDL cholesterol levels, atherosclerosis and/or coronary heart disease. In another embodiment, the disorder is a
25 hyperapobetalipoproteinemia. In a particular embodiment, the individual is an individual at risk for development of a disorder of lipid metabolism.

As used herein, disorders of lipid metabolism include, but are not limited to, abnormal lipoprotein-lipid variability, abnormal LDL-cholesterol levels, abnormal plasma total-apolipoprotein B levels, atherosclerosis and coronary heart disease. In
30 a particular embodiment, the individual is an individual at risk for development of disorders of lipid metabolism. In another embodiment the individual exhibits clinical symptomology associated with disorders of lipid metabolism. In one

embodiment, the individual has been clinically diagnosed as having one or more disorders of lipid metabolism.

In another embodiment, the invention relates to a method for predicting the likelihood that an individual will have a disorder associated with a "G" at nucleotide
5 position 696 of the PPAR α gene (or diagnosing or aiding in the diagnosis of such a disorder) comprising the steps of obtaining a DNA sample from an individual to be assessed and determining the nucleotide present at position 696 of the PPAR α gene. The presence of a "G" at this position indicates that the individual has a greater likelihood of having a disorder associated therewith than an individual having a "C"
10 at that position, or a greater likelihood of having more severe symptomology. Conversely, the presence of a "C" at this position indicates that the individual has a lower likelihood of having a disorder associated therewith than an individual having a "G" at that position, or a greater likelihood of having less severe symptomology. In a preferred embodiment, the disorder is high LDL cholesterol levels, atherosclerosis
15 and/or coronary heart disease. In another embodiment, the disorder is a hyperapobetalipoproteinemia. In a particular embodiment, the individual is an individual at risk for development of such a disorder.

In another embodiment, the invention relates to a method for predicting the likelihood that an individual will have a disorder associated with a "C" at nucleotide
20 position 696 of the PPAR α gene (or diagnosing or aiding in the diagnosis of such a disorder) comprising the steps of obtaining a DNA sample from an individual to be assessed and determining the nucleotide present at position 696 of the PPAR α gene. The presence of a "C" at this position indicates that the individual has a greater likelihood of having a disorder associated therewith than an individual having a "G"
25 at that position, or a greater likelihood of having more severe symptomology. Conversely, the presence of a "G" at this position indicates that the individual has a lower likelihood of having a disorder associated therewith than an individual having a "G" at that position, or a greater likelihood of having less severe symptomology. In a preferred embodiment, the disorder is high LDL cholesterol levels,
30 atherosclerosis and/or coronary heart disease. In another embodiment, the disorder is a hyperapobetalipoproteinemia. In a particular embodiment, the individual is an individual at risk for development of such a disorder.

In another embodiment, the invention relates to a method for predicting the likelihood that an individual will have a disorder associated with an "A" at nucleotide position 971 of the PPAR α gene (or diagnosing or aiding in the diagnosis of such a disorder) comprising the steps of obtaining a DNA sample from an individual to be assessed and determining the nucleotide present at position 971 of the PPAR α gene. The presence of an "A" at this position indicates that the individual has a greater likelihood of having a disorder associated therewith than an individual having a "G" at that position, or a greater likelihood of having more severe symptomology. Conversely, the presence of a "G" at this position indicates that the individual has a lower likelihood of having a disorder associated therewith than an individual having a "A" at that position, or a greater likelihood of having less severe symptomology. In a particular embodiment, the individual is an individual at risk for development of such a disorder.

In another embodiment, the invention relates to a method for predicting the likelihood that an individual will have a disorder associated with a "G" at nucleotide position 971 of the PPAR α gene (or diagnosing or aiding in the diagnosis of such a disorder) comprising the steps of obtaining a DNA sample from an individual to be assessed and determining the nucleotide present at position 971 of the PPAR α gene. The presence of a "G" at this position indicates that the individual has a greater likelihood of having a disorder associated therewith than an individual having a "A" at that position, or a greater likelihood of having more severe symptomology. Conversely, the presence of an "A" at this position indicates that the individual has a lower likelihood of having a disorder associated therewith than an individual having a "G" at that position, or a greater likelihood of having less severe symptomology. In a particular embodiment, the individual is an individual at risk for development of such a disorder.

The genetic material to be assessed can be obtained from any nucleated cell from the individual. For assay of genomic DNA, virtually any biological sample (other than pure red blood cells) is suitable. For example, convenient tissue samples include whole blood, semen, saliva, tears, urine, fecal material, sweat, skin and hair. For assay of cDNA or mRNA, the tissue sample must be obtained from an organ in which the target nucleic acid is expressed. For example, cells from tissues that have

a relatively high level of fatty acid catabolism (such as cells of the liver, kidney, heart and muscle) are suitable sources for obtaining cDNA for the PPAR α gene.

Many of the methods described herein require amplification of DNA from target samples. This can be accomplished by e.g., PCR. See generally PCR

- 5 *Technology: Principles and Applications for DNA Amplification* (ed. H.A. Erlich, Freeman Press, NY, NY, 1992); *PCR Protocols: A Guide to Methods and Applications* (eds. Innis, *et al.*, Academic Press, San Diego, CA, 1990); Mattila *et al.*, *Nucleic Acids Res.* 19, 4967 (1991); Eckert *et al.*, *PCR Methods and Applications* 1, 17 (1991); *PCR* (eds. McPherson *et al.*, IRL Press, Oxford); and
- 10 U.S. Patent 4,683,202.

Other suitable amplification methods include the ligase chain reaction (LCR) (see Wu and Wallace, *Genomics* 4, 560 (1989), Landegren *et al.*, *Science* 241, 1077 (1988), transcription amplification (Kwoh *et al.*, *Proc. Natl. Acad. Sci. USA* 86, 1173 (1989)), and self-sustained sequence replication (Guatelli *et al.*, *Proc. Nat.*

15 *Acad. Sci. USA*, 87, 1874 (1990)) and nucleic acid based sequence amplification (NASBA). The latter two amplification methods involve isothermal reactions based on isothermal transcription, which produce both single stranded RNA (ssRNA) and double stranded DNA (dsDNA) as the amplification products in a ratio of about 30 or 100 to 1, respectively.

- 20 The nucleotide which occupies the polymorphic site of interest (e.g., the first nucleotide in codon 162 of PPAR α) can be identified by a variety methods, such as Southern analysis of genomic DNA; Northern analysis of RNA; denaturing high pressure liquid chromatography (DHPLC); gene isolation and sequencing; hybridization of an allele-specific oligonucleotide with amplified gene products;
- 25 single base extension (SBE); or analysis of the PPAR α protein. In a preferred embodiment, determination of the allelic form of PPAR α is carried out using SBE-FRET methods, or using chip-based oligonucleotide arrays. A sampling of suitable procedures is discussed below in turn.

1. Allele-Specific Probes

- 30 The design and use of allele-specific probes for analyzing polymorphisms is described by e.g., Saiki *et al.*, *Nature* 324, 163-166 (1986); Dattagupta, EP 235,726,

Saiki, WO 89/11548. Allele-specific probes can be designed that hybridize to a segment of target DNA from one individual but do not hybridize to the corresponding segment from another individual due to the presence of different polymorphic forms in the respective segments from the two individuals.

- 5 Hybridization conditions should be sufficiently stringent that there is a significant difference in hybridization intensity between alleles, and preferably an essentially binary response, whereby a probe hybridizes to only one of the alleles.

Hybridizations are usually performed under stringent conditions, for example, at a salt concentration of no more than 1 M and a temperature of at least 25°C. For
10 example, conditions of 5X SSPE (750 mM NaCl, 50 mM NaPhosphate, 5 mM EDTA, pH 7.4) and a temperature of 25-30°C, or equivalent conditions, are suitable for allele-specific probe hybridizations. Equivalent conditions can be determined by varying one or more of the parameters given as an example, as known in the art, while maintaining a similar degree of identity or similarity between the target
15 nucleotide sequence and the primer or probe used.

Some probes are designed to hybridize to a segment of target DNA such that the polymorphic site aligns with a central position (e.g., in a 15-mer at the 7 position; in a 16-mer, at either the 8 or 9 position) of the probe. This design of probe achieves good discrimination in hybridization between different allelic forms.

- 20 Allele-specific probes are often used in pairs, one member of a pair showing a perfect match to a reference form of a target sequence and the other member showing a perfect match to a variant form. Several pairs of probes can then be immobilized on the same support for simultaneous analysis of multiple polymorphisms within the same target sequence.

25 2. Tiling Arrays

The polymorphisms can also be identified by hybridization to nucleic acid arrays, some examples of which are described in WO 95/11995. WO 95/11995 also describes subarrays that are optimized for detection of a variant form of a precharacterized polymorphism. Such a subarray contains probes designed to be
30 complementary to a second reference sequence, which is an allelic variant of the first reference sequence. The second group of probes is designed by the same principles,

except that the probes exhibit complementarity to the second reference sequence. The inclusion of a second group (or further groups) can be particularly useful for analyzing short subsequences of the primary reference sequence in which multiple mutations are expected to occur within a short distance commensurate with the
5 length of the probes (e.g., two or more mutations within 9 to 21 bases).

3. Allele-Specific Primers

An allele-specific primer hybridizes to a site on target DNA overlapping a polymorphism and only primes amplification of an allelic form to which the primer exhibits perfect complementarity. See Gibbs, *Nucleic Acid Res.* 17, 2427-2448
10 (1989). This primer is used in conjunction with a second primer which hybridizes at a distal site. Amplification proceeds from the two primers, resulting in a detectable product which indicates the particular allelic form is present. A control is usually performed with a second pair of primers, one of which shows a single base mismatch at the polymorphic site and the other of which exhibits perfect
15 complementarity to a distal site. The single-base mismatch prevents amplification and no detectable product is formed. The method works best when the mismatch is included in the 3'-most position of the oligonucleotide aligned with the polymorphism because this position is most destabilizing to elongation from the primer (see, e.g., WO 93/22456).

20 4. Direct-Sequencing

The direct analysis of the sequence of polymorphisms of the present invention can be accomplished using either the dideoxy chain termination method or the Maxam Gilbert method (see Sambrook *et al.*, *Molecular Cloning, A Laboratory Manual* (2nd Ed., CSHP, New York 1989); Zyskind *et al.*, *Recombinant DNA*
25 *Laboratory Manual*, (Acad. Press, 1988)).

5. Denaturing Gradient Gel Electrophoresis

Amplification products generated using the polymerase chain reaction can be analyzed by the use of denaturing gradient gel electrophoresis. Different alleles can be identified based on the different sequence-dependent melting properties and

electrophoretic migration of DNA in solution. Erlich, ed., *PCR Technology, Principles and Applications for DNA Amplification*, (W.H. Freeman and Co, New York, 1992), Chapter 7.

6. Single-Strand Conformation Polymorphism Analysis

- 5 Alleles of target sequences can be differentiated using single-strand conformation polymorphism analysis, which identifies base differences by alteration in electrophoretic migration of single stranded PCR products, as described in Orita *et al.*, *Proc. Nat. Acad. Sci.* 86, 2766-2770 (1989). Amplified PCR products can be generated as described above, and heated or otherwise denatured, to form single
10 stranded amplification products. Single-stranded nucleic acids may refold or form secondary structures which are partially dependent on the base sequence. The different electrophoretic mobilities of single-stranded amplification products can be related to base-sequence differences between alleles of target sequences.

7. Single-Base Extension

- 15 An alternative method for identifying and analyzing polymorphisms is based on single-base extension (SBE) of a fluorescently-labeled primer coupled with fluorescence resonance energy transfer (FRET) between the label of the added base and the label of the primer. Typically, the method, such as that described by Chen *et al.*, (*PNAS* 94:10756-61 (1997), incorporated herein by reference) uses a locus-
20 specific oligonucleotide primer labeled on the 5' terminus with 5-carboxyfluorescein (FAM). This labeled primer is designed so that the 3' end is immediately adjacent to the polymorphic site of interest. The labeled primer is hybridized to the locus, and single base extension of the labeled primer is performed with fluorescently labeled dideoxynucleotides (ddNTPs) in dye-terminator sequencing fashion,
25 except that no deoxynucleotides are present. An increase in fluorescence of the added ddNTP in response to excitation at the wavelength of the labeled primer is used to infer the identity of the added nucleotide.

The variant allele described herein may contribute to the bias of an individual toward disorders of lipid metabolism in one or more different ways. The

polymorphism may contribute to phenotype by affecting protein structure as a result of the alteration in amino acid sequence. The polymorphism may exert phenotypic effects indirectly via influence on replication, transcription, and translation. More than one phenotypic trait may be affected. For example, other disorders which are
5 related to disorders of lipid metabolism may also be affected by the PPAR α polymorphism described herein. The polymorphism may also alter the interaction of the gene or gene product with drugs which normally interact directly with PPAR α (e.g., fibrates and other drugs which affect lipid metabolism). Additionally, the described polymorphism may predispose an individual to a distinct mutation that is
10 causally related to a certain phenotype, such as susceptibility or resistance to disorders of lipid metabolism. The discovery of the polymorphism and its correlation with disorders of lipid metabolism facilitates biochemical analysis of the variant and the development of assays to characterize the variant and to screen for pharmaceuticals that interact directly with one or another form of the protein.

15 Alternatively, this particular polymorphism may be one of a group of two or more polymorphisms in the PPAR α gene which contributes to the presence, absence or severity of the disorder of lipid metabolism. An assessment of other polymorphisms within the gene can be undertaken, and the separate and combined effects of these polymorphisms on the disorder phenotype can be assessed.

20 Correlation between a particular phenotype, e.g., the high LDL-cholesterol phenotype, and the presence or absence of a particular allele is performed for a population of individuals who have been tested for the presence or absence of the phenotype. Correlation can be performed by standard statistical methods such as a Chi-squared test and statistically significant correlations between polymorphic
25 form(s) and phenotypic characteristics are noted.

This correlation can be exploited in several ways. In the case of a strong correlation between a particular polymorphic form, e.g., the variant allele for PPAR α , and a disorder for which treatment is available, detection of the polymorphic form in an individual may justify immediate administration of
30 treatment, or at least the institution of regular monitoring of the individual. Detection of a polymorphic form correlated with a disorder in a couple contemplating a family may also be valuable to the couple in their reproductive

decisions. For example, the female partner might elect to undergo *in vitro* fertilization to avoid the possibility of transmitting such a polymorphism from her husband to her offspring. In the case of a weaker, but still statistically significant correlation between a polymorphic form and a particular disorder, immediate
5 therapeutic intervention or monitoring may not be justified. Nevertheless, the individual can be motivated to begin simple life-style changes (e.g., diet, therapy or counseling) that can be accomplished at little cost to the individual but confer potential benefits in reducing the risk of conditions to which the individual may have increased susceptibility by virtue of the particular allele. Furthermore,
10 identification of a polymorphic form correlated with enhanced receptiveness to one of several treatment regimes for a disorder indicates that this treatment regime should be followed for the individual in question.

Furthermore, it may be possible to identify a physical linkage between a genetic locus associated with a trait of interest and polymorphic markers that are not
15 associated with the trait, but are in physical proximity with the genetic locus responsible for the trait and co-segregate with it. Such analysis is useful for mapping a genetic locus associated with a phenotypic trait to a chromosomal position, and thereby cloning gene(s) responsible for the trait. See Lander *et al.*, *Proc. Natl. Acad. Sci. (USA)* 83, 7353-7357 (1986); Lander *et al.*, *Proc. Natl. Acad. Sci. (USA)* 84, 2363-2367 (1987); Donis-Keller *et al.*, *Cell* 51, 319-337 (1987);
20 Lander *et al.*, *Genetics* 121, 185-199 (1989)). Genes localized by linkage can be cloned by a process known as directional cloning. See Wainwright, *Med. J. Australia* 159, 170-174 (1993); Collins, *Nature Genetics* 1, 3-6 (1992).

Linkage studies are typically performed on members of a family. Available
25 members of the family are characterized for the presence or absence of a phenotypic trait and for a set of polymorphic markers. The distribution of polymorphic markers in an informative meiosis is then analyzed to determine which polymorphic markers co-segregate with a phenotypic trait. See, e.g., Kerem *et al.*, *Science* 245, 1073-1080 (1989); Monaco *et al.*, *Nature* 316, 842 (1985); Yamoka *et al.*, *Neurology* 40, 222-
30 226 (1990); Rossiter *et al.*, *FASEB Journal* 5, 21-27 (1991).

Linkage is analyzed by calculation of LOD (log of the odds) values. A lod value is the relative likelihood of obtaining observed segregation data for a marker

- and a genetic locus when the two are located at a recombination fraction θ , versus the situation in which the two are not linked, and thus segregating independently (Thompson & Thompson, *Genetics in Medicine* (5th ed, W.B. Saunders Company, Philadelphia, 1991); Strachan, "Mapping the human genome" in *The Human*
- 5 *Genome* (BIOS Scientific Publishers Ltd, Oxford), Chapter 4). A series of likelihood ratios are calculated at various recombination fractions (θ), ranging from $\theta = 0.0$ (coincident loci) to $\theta = 0.50$ (unlinked). Thus, the likelihood at a given value of θ is: probability of data if loci linked at θ to probability of data if loci unlinked. The computed likelihoods are usually expressed as the \log_{10} of this ratio
- 10 (i.e., a lod score). For example, a lod score of 3 indicates 1000:1 odds against an apparent observed linkage being a coincidence. The use of logarithms allows data collected from different families to be combined by simple addition. Computer programs are available for the calculation of lod scores for differing values of θ (e.g., LIPED, MLINK (Lathrop, *Proc. Nat. Acad. Sci. (USA)* 81, 3443-3446 (1984)).
- 15 For any particular lod score, a recombination fraction may be determined from mathematical tables. See Smith *et al.*, *Mathematical tables for research workers in human genetics* (Churchill, London, 1961); Smith, *Ann. Hum. Genet.* 32, 127-150 (1968). The value of θ at which the lod score is the highest is considered to be the best estimate of the recombination fraction.
- 20 Positive lod score values suggest that the two loci are linked, whereas negative values suggest that linkage is less likely (at that value of θ) than the possibility that the two loci are unlinked. By convention, a combined lod score of +3 or greater (equivalent to greater than 1000:1 odds in favor of linkage) is considered definitive evidence that two loci are linked. Similarly, by convention,
- 25 negative lod score of -2 or less is taken as definitive evidence against linkage of the two loci being compared. Negative linkage data are useful in excluding a chromosome or a segment thereof from consideration. The search focuses on the remaining non-excluded chromosomal locations.

The invention further relates to isolated proteins or polypeptides comprising

30 all or a portion of the variant amino acid sequence of PPAR α (e.g., wherein reference or wildtype PPAR α is exemplified by SEQ ID NO: 2; Figure 3), and to isolated proteins or polypeptides comprising all or a portion of the variant amino

acid sequence of PPAR α . Preferred polypeptides are at least 10 contiguous amino acids and comprise the polymorphic amino acid, e.g., a portion of SEQ ID NO: 2 which is at least 10 contiguous amino acids and comprises the valine at residue 162. The invention further relates to isolated nucleic acid molecules encoding such

5 proteins and polypeptides. In addition, the present invention includes biologically active fragments of the polypeptides, or analogs thereof, including organic molecules which simulate the interactions of the peptides. Biologically active fragments include any portion of the full-length polypeptide which confers a biological function on the gene product, including ligand binding, and antibody

10 binding. Ligand binding includes binding by nucleic acids, proteins or polypeptides, small biologically active molecules, or large cellular structures.

In one embodiment, the invention relates to a method for predicting the likelihood that an individual will have a disorder of lipid metabolism (or diagnosing or aiding in the diagnosis of a disorder of lipid metabolism), e.g., high LDL

15 cholesterol levels, atherosclerosis, coronary heart disease and/or hyperapobetalipoproteinemias, comprising the steps of obtaining a biological sample comprising the PPAR α protein or relevant portion thereof from an individual to be assessed and determining the amino acid present at amino acid position 162 of the PPAR α protein. The presence of a leucine (the reference amino acid) at this

20 position indicates that the individual has a lower likelihood of having a disorder of lipid metabolism than an individual having a valine at that position, or a greater likelihood of having less severe symptomology. In a preferred embodiment, the disorder is high LDL cholesterol levels, atherosclerosis and/or coronary heart disease. In another embodiment, the disorder is a hyperapobetalipoproteinemia. In

25 a particular embodiment, the individual is an individual at risk for development of a disorder of lipid metabolism.

In another embodiment, the invention relates to a method for predicting the likelihood that an individual will have a disorder of lipid metabolism (or diagnosing or aiding in the diagnosis of a disorder of lipid metabolism), e.g., high LDL

30 cholesterol levels, atherosclerosis, coronary heart disease and/or hyperapobetalipoproteinemias, comprising the steps of obtaining a biological sample comprising the PPAR α protein or relevant portion thereof from an individual to be

assessed and determining the amino acid present at amino acid position 162 of the PPAR α protein. The presence of a valine (the variant amino acid) at this position indicates that the individual has a greater likelihood of having a disorder of lipid metabolism than an individual having a leucine at that position, or a greater
5 likelihood of having more severe symptomology. In a preferred embodiment, the disorder is high LDL cholesterol levels, atherosclerosis and/or coronary heart disease. In another embodiment, the disorder is a hyperapobetalipoproteinemia. In a particular embodiment, the individual is an individual at risk for development of a disorder of lipid metabolism.

10 In another embodiment, the invention relates to a method for predicting the likelihood that an individual will have a disorder associated with a valine at amino acid position 162 of the PPAR α protein (or diagnosing or aiding in the diagnosis of such a disorder) comprising the steps of obtaining a biological sample comprising the PPAR α protein or relevant portion thereof from an individual to be assessed and
15 determining the amino acid present at amino acid position 162 of the PPAR α protein. The presence of a valine at this position indicates that the individual has a greater likelihood of having a disorder associated therewith than an individual having a leucine at that position, or a greater likelihood of having more severe symptomology. Conversely, the presence of a leucine at this position indicates that
20 the individual has a lower likelihood of having a disorder associated therewith than an individual having a valine at that position, or a greater likelihood of having less severe symptomology. In a preferred embodiment, the disorder is high LDL cholesterol levels, atherosclerosis and/or coronary heart disease. In another embodiment, the disorder is a hyperapobetalipoproteinemia. In a particular
25 embodiment, the individual is an individual at risk for development of such a disorder.

In another embodiment, the invention relates to a method for predicting the likelihood that an individual will have a disorder associated with a leucine at amino acid position 162 of the PPAR α protein (or diagnosing or aiding in the diagnosis of
30 such a disorder) comprising the steps of obtaining a biological sample comprising the PPAR α protein or relevant portion thereof from an individual to be assessed and determining the amino acid present at amino acid position 162 of the PPAR α

protein. The presence of a leucine at this position indicates that the individual has a greater likelihood of having a disorder associated therewith than an individual having a valine at that position, or a greater likelihood of having more severe symptomology. Conversely, the presence of a valine at this position indicates that
5 the individual has a lower likelihood of having a disorder associated therewith than an individual having a leucine at that position, or a greater likelihood of having less severe symptomology. In a preferred embodiment, the disorder is high LDL cholesterol levels, atherosclerosis and/or coronary heart disease. In another embodiment, the disorder is a hyperapobetalipoproteinemia. In a particular
10 embodiment, the individual is an individual at risk for development of such a disorder.

In these embodiments of the invention, the biological sample contains protein molecules from the test subject. The term "relevant portion" of the protein is intended to mean that the portion of the protein contains the relevant amino acid
15 position to be analysed. *In vitro* techniques for detection of protein include enzyme linked immunosorbent assays (ELISAs), Western blots, immunoprecipitations and immunofluorescence. Furthermore, *in vivo* techniques for detection of protein include introducing into a subject a labeled anti-protein antibody. For example, the antibody can be labeled with a radioactive marker whose presence and location in a
20 subject can be detected by standard imaging techniques. Polyclonal and/or monoclonal antibodies that specifically bind to variant gene products but not to corresponding reference gene products, and vice versa, are also provided. Antibodies can be made by injecting mice or other animals with the variant gene product or synthetic peptide fragments thereof comprising the variant portion.

25 Monoclonal antibodies are screened as are described, for example, in Harlow & Lane, *Antibodies, A Laboratory Manual*, Cold Spring Harbor Press, New York (1988); Goding, *Monoclonal antibodies, Principles and Practice* (2d ed.) Academic Press, New York (1986). Monoclonal antibodies are tested for specific immunoreactivity with a variant gene product and lack of immunoreactivity to the
30 corresponding prototypical gene product. These antibodies are useful in diagnostic assays for detection of the variant form, or as an active ingredient in a pharmaceutical composition.

Polyclonal and/or monoclonal antibodies that specifically bind to variant gene products but not to corresponding reference gene products, or to reference gene products and not to corresponding variant gene products, are also provided.

Antibodies can be made by injecting mice or other animals with the variant gene product or synthetic peptide fragments thereof comprising the variant portion.
5 Monoclonal antibodies are screened as are described, for example, in Harlow & Lane, *Antibodies, A Laboratory Manual*, Cold Spring Harbor Press, New York (1988); Goding, *Monoclonal antibodies, Principles and Practice* (2d ed.) Academic Press, New York (1986). Monoclonal antibodies are tested for specific
10 immunoreactivity with a variant gene product and lack of immunoreactivity to the corresponding prototypical gene product. These antibodies are useful in diagnostic assays for detection of the variant form, or as an active ingredient in a pharmaceutical composition.

The invention further pertains to compositions, e.g., vectors, comprising a
15 nucleotide sequence encoding the reference or variant gene product. For example, variant genes can be expressed in an expression vector in which a variant gene is operably linked to a native or other promoter. Usually, the promoter is a eukaryotic promoter for expression in a mammalian cell. The transcription regulation sequences typically include a heterologous promoter and optionally an enhancer
20 which is recognized by the host. The selection of an appropriate promoter, for example trp, lac, phage promoters, glycolytic enzyme promoters and tRNA promoters, depends on the host selected. Commercially available expression vectors can be used. Vectors can include host-recognized replication systems, amplifiable genes, selectable markers, host sequences useful for insertion into the host genome,
25 and the like.

The means of introducing the expression construct into a host cell varies depending upon the particular construction and the target host. Suitable means include fusion, conjugation, transfection, transduction, electroporation or injection, as described in Sambrook, *supra*. A wide variety of host cells can be employed for
30 expression of the variant gene, both prokaryotic and eukaryotic. Suitable host cells include bacteria such as *E. coli*, yeast, filamentous fungi, insect cells, mammalian cells, typically immortalized, e.g., mouse, CHO, human and monkey cell lines and

derivatives thereof. Preferred host cells are able to process the variant gene product to produce an appropriate mature polypeptide. Processing includes glycosylation, ubiquitination, disulfide bond formation, general post-translational modification, and the like.

5 It is also contemplated that cells can be engineered to express the variant or reference allele of the invention by gene therapy methods. For example, DNA encoding the reference PPAR α gene product, or an active fragment or derivative thereof, can be introduced into an expression vector, such as a viral vector, and the vector can be introduced into appropriate cells in an animal. In such a method, the
10 cell population can be engineered to inducibly or constitutively express active PPAR α gene product. In a preferred embodiment, the vector is delivered to the bone marrow, for example as described in Corey *et al.* (*Science* 244:1275-1281 (1989)).

The invention further provides transgenic nonhuman animals capable of
15 expressing an exogenous reference or variant PPAR α gene and/or having one or both alleles of an endogenous PPAR α gene inactivated. Expression of an exogenous variant gene is usually achieved by operably linking the gene to a promoter and optionally an enhancer, and microinjecting the construct into a zygote. See Hogan *et al.*, "Manipulating the Mouse Embryo, A Laboratory Manual," Cold
20 Spring Harbor Laboratory. Inactivation of endogenous variant genes can be achieved by forming a transgene in which a cloned variant gene is inactivated by insertion of a positive selection marker. See Capecchi, *Science* 244, 1288-1292 (1989). The transgene is then introduced into an embryonic stem cell, where it undergoes homologous recombination with an endogenous variant gene. Mice and
25 other rodents are preferred animals. Such animals provide useful drug screening systems.

In another embodiment, the invention relates to pharmaceutical compositions comprising a reference PPAR α gene product. As used herein, a reference PPAR α gene product is intended to mean functional gene products which are encoded by the
30 reference allele of the PPAR α gene. The invention further relates to the use of compositions (i.e., agonists) which enhance or increase the activity of a peptide comprising the reference PPAR α gene product, or a functional portion thereof, for

use in the treatment of disorders of lipid metabolism. The invention also relates to the use of compositions (i.e., antagonists) which reduce or decrease the activity of a peptide comprising the variant PPAR α gene product, or a functional portion thereof, for use in the treatment of disorders of lipid metabolism.

5 For instance, the polypeptide or protein, or fragment thereof, of the present invention can be formulated with a physiologically acceptable medium to prepare a pharmaceutical composition. The particular physiological medium may include, but is not limited to, water, buffered saline, polyols (e.g., glycerol, propylene glycol, liquid polyethylene glycol) and dextrose solutions. The optimum concentration of
10 the active ingredient(s) in the chosen medium can be determined empirically, according to procedures well known to those skilled in the art and will depend on the ultimate pharmaceutical formulation desired. Methods of introduction of exogenous peptides at the site of treatment include, but are not limited to, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, oral and intranasal. Other
15 suitable methods of introduction can also include rechargeable or biodegradable devices and slow release polymeric devices. The pharmaceutical compositions of this invention can also be administered as part of a combinatorial therapy with other agents and treatment regimens.

A non-limiting description of preferred embodiments of the invention follows.

20 EXAMPLES

Methods

Subjects: Two independently recruited cohorts of unrelated adult Caucasians from the Quebec City area and the Saguenay-Lac-St-Jean (SLSJ) region were used to test the hypothesis that genetic variation in PPAR α may be associated with the
25 development of dyslipidemia.

Type 2 diabetes case-control SLSJ cohort: All individuals included in this cohort are of French Canadian descent above the age of 18 years and were recruited by the Chicoutimi Hospital Lipid Clinic because they presented a family history of dyslipidemia or diabetes mellitus. These participants had previously received a
30 family code which assured the inclusion in the present study of only one patient per

pedigree in order to control for potential inflation of statistical significance due to lack of independence of observations (12). One hundred and twenty-one unrelated patients who received a new diagnosis of type 2 diabetes following an oral glucose tolerance test (OGTT) (67 men and 54 women; diabetic group), were age and sex-matched (1:1) with a patient having a normal OGTT (control group). Type 2 diabetes was defined according to the World Health Organization criteria as a 2-hour glucose concentration ≥ 11.1 mmol/L after a 75g oral glucose absorption, whereas subjects were included in the control group when they had a 2-hour glucose concentration below 7.8 mmol/L (13). Patients diagnosed with type 2 diabetes received dietary counseling according to the American Diabetes Association. Subjects with familial hyperchylomicronemia due to mutations in the lipoprotein lipase gene were excluded from the present study. Subjects were screened for the presence of LDL-receptor gene mutations that cause familial hypercholesterolemia (FH) among French-Canadians. FH screening included two deletions (5 kb and > 15 kb) and point mutations W66G, E207K, C646Y, Y268X and R329X (14-16). Waist and hip circumferences were assessed following the procedures recommended by the Airlie Conference (17). Body weight and height were also recorded, and the BMI was calculated in kg/m^2 . Medication-free lipoprotein profiles were obtained for each subject enrolled in this study. Total-cholesterol, LDL-cholesterol, HDL-cholesterol, total-apolipoprotein B levels and plasma triglyceride concentrations were measured as previously described (18). Biological and lifestyle variables as well as medical and nutritional histories were obtained through questionnaires and physical exams performed at the Chicoutimi Hospital Lipid Clinic by trained nurses, dieticians and physicians. Smoking habits were classified as follows: 1) patients who never smoked; and 2) patients who ever smoked. Alcohol consumption was treated in two categories: 1) regular drinkers (>5 ounces of absolute alcohol/week); and 2) non-regular drinkers. A subject was considered as hypertensive when diagnosis of essential hypertension had been previously established or when three values of systolic ≥ 140 mmHg or diastolic ≥ 90 mmHg blood pressure had been recorded in the patient's medical chart. This project has been approved by the Chicoutimi Hospital Ethic Committee.

Quebec City non-diabetic male cohort: This cohort included 193 men who were recruited from the Quebec City area by solicitation through the media and were selected to cover a wide range of visceral adipose tissue accumulation. All subjects were sedentary, non-smokers and free from metabolic disorders requiring treatment
5 such as diabetes, hypertension and coronary heart disease. A high alcohol consumption was also an exclusion criteria. BMI and waist circumference were measured as described above. Blood samples were obtained after an overnight fast from an antecubital vein. Cholesterol and triglyceride concentrations in plasma and lipoprotein fractions were enzymatically measured on an Analyzer Technicon RA-
10 500 (Bayer Corporation, Tarry Town, NY). VLDL ($d < 1.006$ g/ml) were isolated by ultracentrifugation (19) and the HDL fraction was obtained after precipitation of LDL in the infranatant ($d > 1.006$ g/ml) with heparin and $MnCl_2$ (20). The cholesterol content of HDL₂-chol and HDL₃-chol subfractions prepared by a precipitation method was also determined (21). HDL-apoAI was measured in the
15 infranatant ($d > 1.006$ g/ml) by the rocket immunoelectrophoretic method of Laurell as previously described (22). The serum standards were prepared in the laboratory, calibrated against reference sera from the Centers for Disease Control (Atlanta, GA), lyophilized, and stored at $-85^{\circ}C$ until use. Coefficients of variation for repeated measurements of HDL-cholesterol and apoAI concentrations were 3.3 and 3.4%,
20 respectively.

Genomic structure of the PPAR α gene: In order to design intronic primers for the amplification of each exon, genomic sequences were sought for the intronic regions surrounding all PPAR α exons. To do so, the mRNA sequence of PPAR α (23) was compared with a contiguous genomic DNA region taken from sequences of three
25 overlapping clones from GenBank (Accession number: Z94161.5, AL049856, AL078611). Intronic primers were then designed using the Primer 3.0 software available on the Whitehead Institute/MIT Center for Genome Research server (<http://www.genome.wi.mit.edu/cgi-bin/primer/primer3.cgi>).

PCR amplification of PPAR α exons and sequencing: All exons and exon-intron
30 boundaries were amplified from genomic DNA by use of specific primers derived

from the 5' and 3' intronic sequences (Table 1). The annealing temperature for all primer pairs was 59°C. PCR conditions were as follows: reaction volume was of 50 µl, 1.25 unit AmpliTaq Gold polymerase (Perkin-Elmer Cetus) in the buffer recommended by the manufacturer; 2.5 mM MgCl₂; 0.2 mM dNTPs; primers at a final concentration of 0.5 µM and 100 ng of template genomic DNA. PCR products were purified with magnetic beads BioMag DNA Sep (PerSeptive Biosystems, Framingham, MA). Sequencing reactions were performed using BigDye Primer Cycle sequencing ready reactions -21M13 kit (PE Applied BioSystems, Foster City, CA) and were analyzed on an ABI 377 automated sequencer (PE Applied BioSystems, Foster City, CA). The gel files were processed using the Sequencing analysis software (PE Applied BioSystems, Foster City, CA).

Detection of the L162V by polymerase chain reaction, restriction fragment length polymorphism (PCR-RFLP): The L162V mutation is caused by a C→ G transversion at nucleotide 484 in exon 5. It does not alter any restriction site; thus, mismatch PCR approach was performed with the following primers Ex5.F(2) 5'-GACTCAAGCTGGTGTATGACAAGT-3' (SEQ ID NO: 3) and Ex5.R-mismatch 5'-CGTTGTGTGACATCCCGACAGAAT-3' (SEQ ID NO: 4). PCR conditions were as described above. PCR products were digested with HinfI, electrophoresed on either 12% acrylamide or 4% agarose gel and stained with ethidium bromide.

Statistical analysis: Allelic frequencies were compared using Chi-square analyses. Differences between genotypic groups were tested for statistical significance by using Student's test, and as indicated in Tables 4 and 5, some variables were log₁₀ transformed in order to normalize their distributions. Before performing statistical analyses on lipoprotein-lipid variables of the type 2 diabetes case-control cohort, 27 heterozygous FH subjects were excluded. Analysis of covariance was used to adjust lipid, lipoprotein and apolipoprotein variables for age alone or for both age and BMI or age and waist circumference. All statistical analyses were performed using SPSS package (SPCC Inc.) or JMP statistical package from SAS institute.

RESULTS

Structure of the human PPAR α gene: The structural organization of the gene was determined by comparing the human PPAR α mRNA sequence with genomic sequences obtained from overlapping clones (Figure 1). All 5' donor and 3' acceptor splice sites were found to obey the gt...ag rule (Table 2). Furthermore, all exon-intron boundaries were confirmed by direct sequencing of each exon using primers derived from intronic sequences. The human PPAR α gene spans approximately 83.7Kb. As it is the case for the mouse, human PPAR α gene mRNA is composed of 8 exons, with a 5' untranslated region encoded by exons 1, 2 and part of exon 3. The coding region of PPAR α is comprised within the remaining 6 exons. Intron lengths vary from 1.4 to 24.8 Kb as indicated in Figure 1.

Identification of polymorphisms in the human PPAR α gene: All exons and the exon-intron splicing boundaries of PPAR α were screened by sequencing 12 patients who have been diagnosed with type 2 diabetes and 2 controls. All the amplified fragments of the PPAR α gene showed the expected length, suggesting the absence of deletions, duplications or rearrangements within these fragments. Two polymorphisms were discovered within the exons. Based on the predicted amino acid sequence for this gene, the polymorphism in exon 7 is silent, with a G to A substitution which does not alter the threonine at codon 253. A new polymorphism was discovered in exon 5, which results in a L162 V missense mutation. Specifically, the C to G transversion in the first position of codon 162 leads to a substitution of a valine for a leucine.

Genotype determination of the L162V mutation: A rapid screening test was developed using the mismatch PCR approach to genotype the L162V polymorphism. Using the primers Ex5R-mismatch and Ex5.F(2) described in the methods, PCR products from the mutant allele contain an *Hinf*I restriction site that is abolished in the presence of the C at position 484. After digestion with *Hinf*I, the PCR product from the normal allele results in a 117 bp fragment, whereas it is cleaved into 93 bp and 24 bp fragments with the mutant allele. Genotypes obtained by the mismatch PCR approach and by direct sequencing of exon 5 were in perfect

agreement. Moreover, genotypes obtained by the mismatch PCR approach were repeated for a minimum of 60 randomly chosen individuals, and the reproducibility was 100%.

Association between the L162V missense mutation and lipoprotein-lipid

5 **concentrations:** Chi-square analysis performed independently within the SLSJ control and diabetic groups revealed that confounding factors such as the smoking status, alcohol consumption and the use of beta-blockers or diuretic drugs as well as the prevalence of hypertension were similar between the two PPAR α genotypic groups. Furthermore, since patients have been assigned to the diabetic or control
10 group after the OGTT, medication-free lipoprotein-lipid profile as well as fasting blood glucose and insulin levels obtained at the OGTT are shown. Subjects' characteristics according to the L162V genotype are shown in Table 4. Since mutations in the LDL-receptor gene that cause FH have a dramatic effect on the lipoprotein concentrations, heterozygous FH subjects were excluded prior to
15 statistical analyses. Carriers of the rare V162 and L162 homozygotes had similar fasting glucose and insulin levels as well as plasma HDL-cholesterol concentrations, independent of their clinical status: diabetic or control. Although, both diabetics and controls tended to have higher LDL-cholesterol and total-apolipoprotein B levels when they were carriers of the V162 allele, the difference did not reach
20 statistical significance. After adjustments for co-variables such as age and/or BMI, results remained unchanged.

Although not significant, the 9% increase in plasma LDL-cholesterol levels observed among V162 carriers encouraged further investigation of the effect of that missense mutation in a larger cohort. For this purpose, a cohort of 193 non-diabetic
25 males recruited in the greater Quebec City area were screened and for whom a more detailed lipoprotein-lipid profile was obtained. Furthermore, these subjects were free of metabolic disorders requiring treatment such as type 2 diabetes, hypertension or coronary heart disease. In the Quebec City non-diabetic male cohort, the frequency of the rare V162 allele was 6.6% (Table 3). Comparison of the
30 lipoprotein lipid profile between the two genotypic groups showed that as in the diabetes case-control cohort, subjects carrying the rare V162 allele had higher LDL-

cholesterol, LDL-apolipoprotein B and total-apolipoprotein B levels than L162 homozygotes, and these differences reach statistical significance (Table 5). Importantly, these differences remained significant after adjustments for age and BMI or for age and waist circumference.

- 5 While this invention has been particularly shown and described with references to preferred embodiments thereof, it will be understood by those skilled in the art that various changes in form and details may be made therein without departing from the scope of the invention encompassed by the appended claims.

Table 1: PCR primers for genomic amplification of PPAR α exons.

Exons	Oligonucleotides	Product size
Ex. 1	PPAR α ex1.F 5'-TGTAACCGACGGCCAGTCCATCTGGAACAGTAAATTAAACCC-3' PPAR α ex1.R 5'-GCATCCAGAGGAAACAACCGTAA-3'	169bp
Ex. 2	PPAR α ex2.F 5'-TGTAACCGACGGCCAGTAAATGATTAACAGAAATTCATCCACCA-3' PPAR α ex2.R 5'-TCCATTCAAGCTGCTATAACAAAAT-3'	213bp
Ex. 3	PPAR α ex3.F 5'-TGTAACCGACGGCCAGTCGTCAGTCTTACCAATTGTTCCCT-3' PPAR α ex3.R 5'-AAACTTTCTAGGAAACGGGCACA-3'	311bp
Ex. 4	PPAR α ex4.F 5'-TGTAACCGACGGCCAGTCGTAATGCGAATCACAATCCCTC-3' PPAR α ex4.R 5'-TAAGTAGTTGATGTTGGCGG-3'	300bp
Ex. 5	PPAR α ex5.F 5'-TGTAACCGACGGCCAGTAGTAAGCAAGTGCGCTGGT-3' PPAR α ex5.R 5'-AAGGAAAGGGAACTGAGGAA-3'	243bp
Ex. 6	PPAR α ex6.F 5'-TGTAACCGACGGCCAGTCTCAGTCTGCTGCTGCTGCT-3' PPAR α ex6.R 5'-CCAGAGGAAACCCAGAACAGC-3'	274bp
Ex. 7	PPAR α ex7.F 5'-TGTAACCGACGGCCAGTGCATCCCAATCACCCTGAC-3' PPAR α ex7.R 5'-TCAGTGAACATGATACCAAGCA-3'	530bp
Ex. 8	PPAR α ex8.F 5'-TGTAACCGACGGCCAGTGAATAGCAATCTTGGGTGA-3' PPAR α ex8.R 5'-ACCATGAGCATAATTCGCC-3'	566bp

M13 tail inserted into forward primers is indicated in bold.

Table 2: Exon-Intron splicing boundaries.

Exon (bp)	Donor.....Acceptor	Exon
exon 1 (87)	CAG:gtaa.....acag:TTC	exon 2
exon 2 (84)	GAG:gtag.....ccag:TAG	exon 3
exon 3 (250)	CGG:gtaa.....ccag:ACA	exon 4
exon 4 (161)	AAG:gtag.....acag:GGC	exon 5
exon 5 (139)	ACG:gtag.....ctag:CGA	exon 6
exon 6 (203)	CCA:gtag.....gtag:CCT	exon 7
exon 7 (448)	GAG:gtag.....ctag:ATC	exon 8
exon 8 (480)	TACTGAgTtc	

Nucleotides in exons are indicated in uppercase letters, whereas the flanking nucleotides in introns are in lower case. The exact length in base pairs of the exons is indicated in parenthesis.

Table 3: PPAR α allele frequencies.

Population	L162	V162
Study sample # 1 from SLSJ		
- Diabetic patients	213 (88.0%)	29 (12.0%)
- Non-diabetic patients	211 (87.2%)	31 (12.8%)
Study sample # 2, Quebec city non-diabetic men	370 (93.4%)	26 (6.6%)
Number of alleles and percentage in parenthesis.		

Table 4: Subjects' characteristics according to the L162V genotype in the study sample # 1 of type 2 diabetic/non-diabetic patients.

Variable	Cases			Controls		
	L/L ¹	L/V ²	p-value	L/L ³	L/V ⁴	p-value
Age, years	53.7±6.7 (85)	53.5±8.0 (26)	0.87	52.5±8.5 (76)	52.5±5.9 (28)	0.99
BMI ⁵ , kg/m ²	29.4±4.2 (85)	28.4±5.2 (26)	0.22	28.3±3.8 (76)	29.2±3.7 (28)	0.22
Waist circumference, cm	98.9±11.7 (80)	94.9±11.6 (26)	0.13	92.5±12.1 (76)	96.2±12.3 (28)	0.16
Fasting glucose ⁶ , mmol/L	6.52±1.95 (86)	6.35±1.16 (26)	0.89	5.19±0.55 (76)	5.15±0.67 (28)	0.71
Fasting Insulin ⁷ , pmol/L	127.8±115.8 (43)	147.0±111.0 (15)	0.44	140.4±116.4 (49)	120.0±91.8 (18)	0.74
Total-cholesterol ⁸ , mmol/L	6.53±1.67 (86)	6.71±1.19 (26)	0.42	6.58±1.43 (76)	6.76±1.59 (28)	0.68
LDL-cholesterol ⁹ , mmol/L	4.15±1.35 (62)	4.53±0.85 (21)	0.11	4.19±0.99 (65)	4.57±1.47 (21)	0.43
HDL-cholesterol ¹⁰ , mmol/L	0.93±0.32 (81)	0.97±0.27 (26)	0.38	1.04±0.38 (74)	1.02±0.39 (27)	0.85
Triglycerides ¹¹ , mmol/L	4.19±3.72 (85)	3.37±2.42 (26)	0.38	2.89±2.39 (76)	3.24±2.90 (28)	0.76
Total apolipoprotein B, g/L	1.20±0.22 (61)	1.29±0.21 (18)	0.12	1.19±0.30 (58)	1.28±0.32 (21)	0.22

¹Log₁₀-transformed variables. Data are mean±SD. ²One homozygote V/V included in the L/V group. Number of subjects is shown in parenthesis. Prior statistical analyses, FH patients were excluded. The male/female ratio was of 48/37, 14/12, 41/35 and 18/10 respectively for groups 1 through 4, group numbers are identified by superscripts.

Table 5: Subjects' characteristics according to the L162V genotype in the study sample # 2 of non-diabetic men.

Variable	L/L	L/V	p-value
Age, years	41.7±8.1 (172)	44.7±7.8 (26)	0.08
BMI, kg/m ²	29.9±4.3 (171)	29.1±2.9 (25)	0.36
Waist circumference, cm	101.0±10.7 (171)	99.7±8.9 (26)	0.54
Fasting glucose*, mmol/L	5.41±0.56 (170)	5.33±0.33 (26)	0.55
Fasting Insulin*, pmol/L	109.9±83.7 (170)	85.8±42.5 (26)	0.14
Total-cholesterol, mmol/L	5.21±0.79 (169)	5.50±0.58 (25)	0.08
LDL-cholesterol, mmol/L	3.49±0.74 (169)	3.86±0.56 (25)	0.02
HDL-cholesterol*, mmol/L	0.93±0.20 (169)	0.91±0.17 (25)	0.70
HDL ₂ -cholesterol, mmol/L	0.26±0.16 (166)	0.27±0.17 (25)	0.78
Triglycerides*, mmol/L	2.17±1.16 (169)	1.98±0.80 (25)	0.66
Total-apolipoprotein B, g/L	1.07±0.24 (166)	1.18±0.16 (25)	0.02
LDL-apolipoprotein B, g/L	0.94±0.20 (166)	1.05±0.13 (25)	0.01

* Log₁₀-transformed variables. Data are mean±SD. Number of subjects is shown in parenthesis.

REFERENCES

1. Schoonjans K, Martin G, Staels B, Auwerx J. Peroxisome proliferator-activated receptors, orphans with ligands and functions. *Curr Opin Lipidol.* 1997;8:159-166
2. Schoonjans K, Staels B, Auwerx J. The peroxisome proliferator activated receptors (PPARS) and their effects on lipid metabolism and adipocyte differentiation. *Biochim Biophys Acta* 1996;1302:93-109
3. Schoonjans K, Staels B, Auwerx J. Role of the peroxisome proliferator-activated receptor (PPAR) in mediating the effects of fibrates and fatty acids on gene expression. *J Lipid Res* 1996;37:907-925
4. Vidal-Puig AJ, Considine RV, Jimenez-Linan M, et al. Peroxisome proliferator-activated receptor gene expression in human tissues. Effects of obesity, weight loss, and regulation by insulin and glucocorticoids. *J Clin Invest* 1997;99:2416-2422
5. Brun RP, Tontonoz P, Forman BM, et al. Differential activation of adipogenesis by multiple PPAR isoforms. *Genes Dev.* 1996;10:974-984
6. Isseman I, Green S. Activation of a member of the steroid hormone receptor superfamily by peroxisome proliferators. *Nature* 1990;347:645-650
7. Auboeuf D, Rieusset J, Fajas L, et al. Tissue distribution and quantification of the expression of mRNAs of peroxisome proliferator-activated receptors and liver X receptor-alpha in humans: no alteration in adipose tissue of obese and NIDDM patients. *Diabetes* 1997;46:1319-1327
8. Kliewer SA, Sundseth SS, Jones SA, et al. Fatty acids and eicosanoids regulate gene expression through direct interactions with peroxisome proliferator-activated receptors alpha and gamma. *Proc Natl Acad Sci U.S.A* 1997;94:4318-4323
9. Inoue I, Noji S, Shen MZ, Takahashi K, Katayama S. The peroxisome proliferator-activated receptor alpha (PPAR alpha) regulates the plasma thiobarbituric acid-reactive substance (TBARS) level. *Biochem Biophys Res Commun.* 1997;237:606-610
10. Horrobin DF. Abnormal membrane concentrations of 20 and 22-carbon essential fatty acids: a common link between risk factors and coronary and peripheral vascular disease? *Prostaglandins Leukot Essent Fatty Acids.* 1995;53:385-396
11. Reaven GM. Syndrome X: 6 years later. *J Intern Med Suppl.* 1994;736:13-22:13-22
12. Gaudet D, Arsenault S, Belanger C, et al. Procedure to protect confidentiality of familial data in community genetics and genomic research [In Process Citation]. *Clin Genet* 1999;55:259-264

13. Report on the Expert Committee on the diagnosis and classification of diabetes mellitus. *Diabetes Care* 1997;20:1183-1197
14. Couture P, Vohl MC, Gagné C, et al. Identification of three mutations in the low-density lipoprotein receptor gene causing familial hypercholesterolemia among French-Canadians. *Hum.Mutat.* 1997;suppl 1:s226-s231
15. Ma Y, Bétard C, Roy M, Davignon J, Kessling A. Identification of a second "French Canadian" LDL receptor gene deletion and development of a rapid method to detect both deletions. *Clinical Genetics* 1989;36:219-228
16. Vohl MC, Couture P, Moorjani S, et al. Rapid restriction fragment analysis for screening four point mutations of the low-density lipoprotein receptor gene in French Canadians. *Hum.Mutat.* 1995;6:243-246
17. Standardization of anthropometric measurements. In: *The Airlie (VA) Consensus Conference*, edited by Lohman, T., Roche, A., Martorel, R. Human Kinetics Publishers, 1988;39-80.
18. Gaudet D, Vohl MC, Julien P, et al. Relative contribution of low-density lipoprotein receptor and lipoprotein lipase gene mutations to angiographically assessed coronary artery disease among French Canadians. *Am J Cardiol* 1998;82:299-305
19. Havel RJ, Eder HA, Bragdon JH. The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. *J Clin Invest* 1955;34:1345-1354
20. Burnstein M, Samaille J. Sur un dosage rapide du cholestérol lié aux alpha et aux bêta lipoprotéines du sérum. *Clin Chem Acta* 1960;5:309
21. Gidez LI, Miller GJ, Burnstein M, Slagle S, Eder HA. Separation and quantitation of subclasses of human plasma high density lipoproteins by a simple precipitation procedure. *J.Lipid Res.* 1982;23:1206-1223
22. Laurell CB. Quantitative estimation of proteins by electrophoresis in agarose gel containing antibodies. *Anal Biochem* 1966;15:42
23. Mukherjee R, Jow L, Noonan D, McDonnell DP. Human and rat peroxisome proliferator activated receptors (PPARs) demonstrate similar tissue distribution but different responsiveness to PPAR activators. *J Steroid Biochem.Mol.Biol* 1994;51:157-166
24. Sher T, Yi HF, McBride OW, Gonzalez FJ. cDNA cloning, chromosomal mapping, and functional characterization of the human peroxisome proliferator activated receptor. *Biochem* 1993;32:5598-5604
25. Gearing KL, Crickmore A, Gustafsson JA. Structure of the mouse peroxisome proliferator activated receptor alpha gene. *Biochem.Biophys.Res.Commun.* 1994;199:255-263

26. Tugwood JD, Aldridge TC, Lambe KG, Macdonald N, Woodyatt NJ. Peroxisome proliferator-activated receptors: structures and function. *Ann.N.Y.Acad.Sci.* 1996;804:252-65:252-265
27. Holden PR, Tugwood JD. Peroxisome proliferator-activated receptor alpha: role in rodent liver cancer and species differences. *J Mol.Endocrinol.* 1999;22:1-8
28. Castelli WP, Garrison RJ, Wilson PWF, Abbot RD, Kalousdian S, Kannel WB. Incidence of coronary heart disease and lipoprotein cholesterol levels: the Framingham study. *JAMA* 1986;256:2835-2838
29. Castelli WP, Anderson K, Wilson PW, Levy D. Lipids and risk of coronary heart disease. The Framingham Study. *Ann.Epidemiol.* 1992;2:23-28
30. Lamarche B, Tchernof A, Mauriege P, et al. Fasting insulin and apolipoprotein B levels and low-density lipoprotein particle size as risk factors for ischemic heart disease. *JAMA* 1998;279:1955-1961
31. Bolibar I, Thompson SG, von Eckardstein A, Sandkamp M, Assmann G. Dose-response relationships of serum lipid measurements with the extent of coronary stenosis. Strong, independent, and comprehensive. ECAT Angina Pectoris Study Group. *Arterioscler.Thromb.Vasc.Biol.* 1995;15:1035-1042
32. Djouadi F, Weinheimer CJ, Saffitz JE, et al. A gender-related defect in lipid metabolism and glucose homeostasis in peroxisome proliferator- activated receptor alpha-deficient mice. *J Clin.Invest.* 1998;102:1083-1091
33. Peters JM, Hennuyer N, Staels B, et al. Alterations in lipoprotein metabolism in peroxisome proliferator- activated receptor alpha-deficient mice. *J Biol.Chem.* 1997;272:27307-27312
34. Lamb RG, Koch JC, Bush SR. An enzymatic explanation of the differential effects of oleate and gemfibrozil on cultured hepatocyte triacylglycerol and phosphatidylcholine biosynthesis and secretion. *Biochim.Biophys.Acta* 1993;1165:299-305
35. Ruotolo G, Ericsson CG, Terramanti C, et al. Treatment effects on serum lipoprotein lipids, apolipoproteins and low density lipoprotein particle size and relationships of lipoprotein variables to progression of coronary artery disease in the Bezafibrate Coronary Atherosclerosis Intervention Trial (BECAIT). *J Am Coll.Cardiol.* 1998;32:1648-1656
36. Ericsson CG. Results of the Bezafibrate Coronary Atherosclerosis Intervention Trial (BECAIT) and an update on trials now in progress. *Eur.Heart J* 1998;19 Suppl H:H37-H41

37. de Faire U, Ericsson CG, Grip L, Nilsson J, Svane B, Hamsten A. Retardation of coronary atherosclerosis: the Bezafibrate Coronary Atherosclerosis Intervention Trial (BECAIT) and other angiographic trials. *Cardiovasc.Drugs Ther.* 1997;11 Suppl 1:257-63:257-263
38. Bell AR, Savory R, Horley NJ, et al. Molecular basis of non-responsiveness to peroxisome proliferators: the guinea-pig PPARalpha is functional and mediates peroxisome proliferator- induced hypolipidaemia. *Biochem.J* 1998;332:689-693
39. Gottlicher M, Widmark E, Li Q, Gustafsson JA. Fatty acids activate a chimera of the clofibrate acid-activated receptor and the glucocorticoid receptor. *Proc.Natl.Acad.Sci.U.S.A.* 1992;89:4653-4657

CLAIMS

What is claimed is:

1. A method for predicting the likelihood that an individual will have a disorder of lipid metabolism, comprising the steps of obtaining a DNA sample from an individual to be assessed and determining the nucleotide present at the first position of codon 162 of the PPAR α gene, wherein the presence of a C at this position indicates that the individual has a lower likelihood of having a disorder of lipid metabolism than an individual having a G at that position, or a greater likelihood of having less severe symptomology.
2. A method for predicting the likelihood that an individual will have a disorder of lipid metabolism, comprising the steps of obtaining a DNA sample from an individual to be assessed and determining the nucleotide present at the first position of codon 162 of the PPAR α gene, wherein the presence of a G at this position indicates that the individual has a greater likelihood of having a disorder of lipid metabolism than an individual having a C at that position, or a greater likelihood of having more severe symptomology.
3. A method according to Claim 1 or 2, wherein the disorder is selected from the group consisting of abnormal lipoprotein-lipid variability, abnormal LDL-cholesterol levels, abnormal plasma total-apolipoprotein B levels, atherosclerosis, hyperapobetalipoproteinemia and coronary heart disease.
4. A method for predicting the likelihood that an individual will have a disorder associated with a G at nucleotide position 696 of the PPAR α gene comprising the steps of obtaining a DNA sample from an individual to be assessed and determining the nucleotide present at position 696 of the PPAR α gene, wherein the presence of a G at this position indicates that the individual has a greater likelihood of having a disorder associated therewith than an individual

having a C at that position, or a greater likelihood of having more severe symptomology.

5. A method for predicting the likelihood that an individual will have a disorder associated with a C at nucleotide position 696 of the PPAR α gene comprising
5 the steps of obtaining a DNA sample from an individual to be assessed and determining the nucleotide present at position 696 of the PPAR α gene, wherein the presence of a C at this position indicates that the individual has a greater likelihood of having a disorder associated therewith than an individual having a G at that position, or a greater likelihood of having more severe
10 symptomology.
6. A method for predicting the likelihood that an individual will have a disorder associated with an A at nucleotide position 971 of the PPAR α gene comprising the steps of obtaining a DNA sample from an individual to be assessed and determining the nucleotide present at position 971 of the PPAR α
15 gene, wherein the presence of an A at this position indicates that the individual has a greater likelihood of having a disorder associated therewith than an individual having a G at that position, or a greater likelihood of having more severe symptomology.
7. A method for predicting the likelihood that an individual will have a disorder associated with a G at nucleotide position 971 of the PPAR α gene comprising
20 the steps of obtaining a DNA sample from an individual to be assessed and determining the nucleotide present at position 971 of the PPAR α gene, wherein the presence of a G at this position indicates that the individual has a greater likelihood of having a disorder associated therewith than an individual
25 having a A at that position, or a greater likelihood of having more severe symptomology.
8. A method for predicting the likelihood that an individual will have a disorder of lipid metabolism, comprising the steps of obtaining a biological sample

comprising the PPAR α protein or relevant portion thereof from an individual to be assessed and determining the amino acid present at amino acid position 162 of the PPAR α protein, wherein the presence of a leucine at this position indicates that the individual has a lower likelihood of having a disorder of lipid metabolism than an individual having a valine at that position, or a greater likelihood of having less severe symptomology.

9. A method for predicting the likelihood that an individual will have a disorder of lipid metabolism, comprising the steps of obtaining a biological sample comprising the PPAR α protein or relevant portion thereof from an individual to be assessed and determining the amino acid present at amino acid position 162 of the PPAR α protein, wherein the presence of a valine at this position indicates that the individual has a greater likelihood of having a disorder of lipid metabolism than an individual having a leucine at that position, or a greater likelihood of having more severe symptomology.

10. A method for predicting the likelihood that an individual will have a disorder associated with a valine at amino acid position 162 of the PPAR α protein comprising the steps of obtaining a biological sample comprising the PPAR α protein or relevant portion thereof from an individual to be assessed and determining the amino acid present at amino acid position 162 of the PPAR α protein, wherein the presence of a valine at this position indicates that the individual has a greater likelihood of having a disorder associated therewith than an individual having a leucine at that position, or a greater likelihood of having more severe symptomology.

11. A method for predicting the likelihood that an individual will have a disorder associated with a leucine at amino acid position 162 of the PPAR α protein, comprising the steps of obtaining a biological sample comprising the PPAR α protein or relevant portion thereof from an individual to be assessed and determining the amino acid present at amino acid position 162 of the PPAR α protein, wherein the presence of a leucine at this position indicates that the

individual has a greater likelihood of having a disorder associated therewith than an individual having a valine at that position, or a greater likelihood of having more severe symptomology.

12. A pharmaceutical composition comprising a peptide comprising a functional
5 portion of PPAR α reference gene product for use in the treatment of disorders of lipid metabolism.
13. A method of treating a disorder of lipid metabolism, comprising administering to an individual in need thereof a pharmaceutical composition comprising a peptide comprising a functional portion of PPAR α reference gene product.
- 10 14. A pharmaceutical composition comprising a nucleic acid molecule encoding a peptide comprising a functional portion of PPAR α reference gene product for use in the treatment of disorders of lipid metabolism.
- 15 15. A method of treating a disorder of lipid metabolism, comprising administering to an individual in need thereof a pharmaceutical composition comprising a nucleic acid molecule encoding a peptide comprising a functional portion of PPAR α reference gene product.
16. A method of treating a disorder of lipid metabolism, comprising administering to an individual in need thereof a pharmaceutical composition comprising an agonist of a peptide comprising a functional portion of PPAR α reference gene
20 product.
17. An isolated nucleic acid molecule comprising all or a portion of the variant allele of PPAR α .
18. An isolated nucleic acid molecule according to Claim 17, wherein wildtype PPAR α is exemplified by SEQ ID NO: 1.

-48-

19. An isolated gene product which is encoded by a nucleic acid molecule according to Claim 17.
20. An isolated polypeptide comprising all or a portion of the variant amino acid sequence of PPAR α .
- 5 21. An isolated polypeptide according to Claim 20, wherein wildtype PPAR α is exemplified by SEQ ID NO: 2.

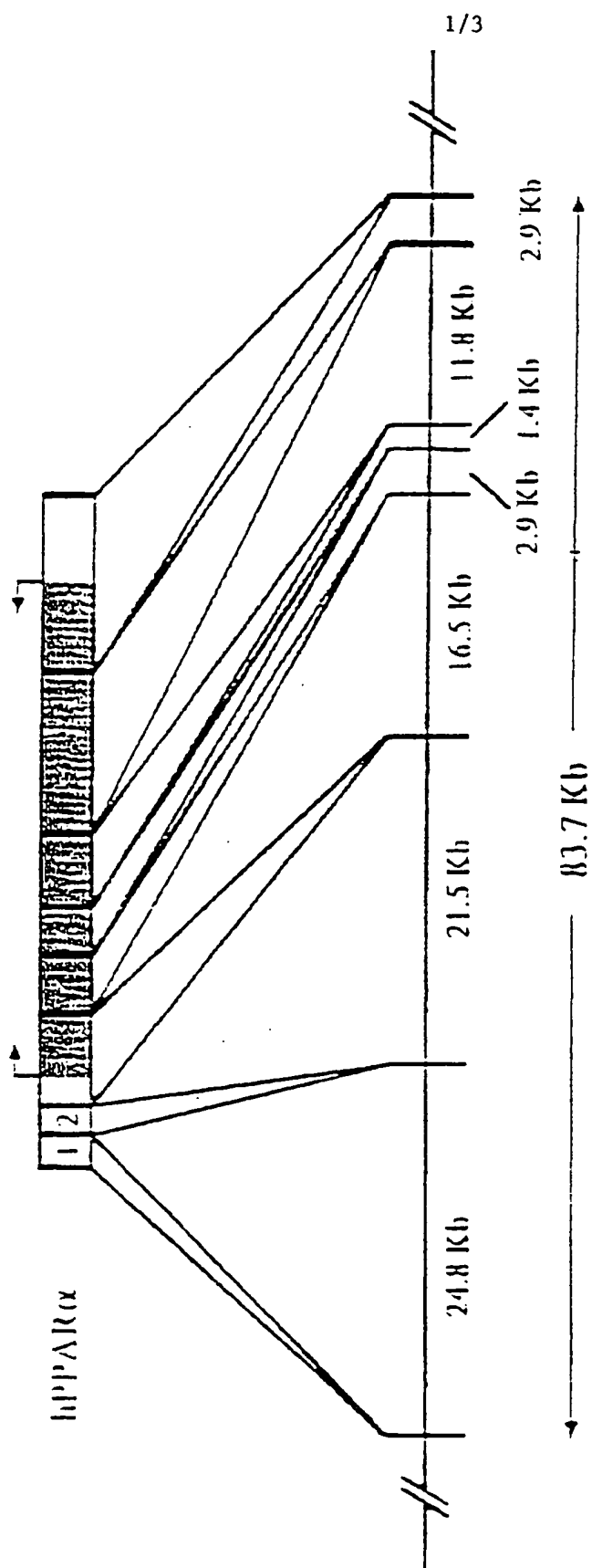


Figure 1

2/3

```

ggcccaggct gaagctcagg gccctgtctg ctctgtggac tcaacagttt gtggcaagac
aagctcagaa ctgagaagct gtcaccacag ttctggaggc tgggaagttc aagatcaaag
tgccagcaga ttcagtgtca tgtgaggacg tgcttcctgc ttcatagata agagcttgga
gctcggcgca caaccagcac catctggctg cgatggtgga cacggaagc ccactctgcc
ccctctcccc actcaggscg gccgatctag agagcccgtt atctgaagag ttcttgcaag
aaatgggaaa catccaagag atttcgcaat ccacggcgga ggatagttct ggaagctttg
gctttacgga ataccagtat ttaggaagct gtcctggctc agatggctcg gtcatacagg
acacgctttc accagcttcg agccctctct cggtgactta tcctgtggct cccggcagcg
tggaacgagtc tcccagtgga gcattgaaca tcgaatgtag aatctgcggg gacaaggcct
caggctatca ttacggagtc cacgcgtgtg aaggctgcaa gggcttctct cggcgaacga
ttcgactcaa gctggtgtat gacaagtgcg accgcagctg caagatccag aaaaagaaca
gaaacaaatg ccagtattgt cgatttcaca agtgcccttc tgtcgggatg tcacacaacg
cgatccgctt tggacgaatg ccaagatctg agaaagcaaa actgaaagca gaaattctta
cctgtgaaca tgacatagaa gattctgaaa ctgcagatct caaatctctg gccaaagaga
tctacgaggc ctacttgaag aacttcaaca tgaacaaggt caaagcccg gtcactctct
caggaaaggc cagtaacaat ccaccttttg tcatacatga tatggagaca ctgtgtatgg
ctgagaagac gctggtggcc aagctggtgg ccaatggcat ccagaacaag gaggcggagg
tcgcacatct tcactgctgc cagtgcacgt cagtggagac cgtcacggag ctcacggaat
tcgccaaggc catcccaggc ttcgcaaaact tggacctgaa cgatcaagtg acattgctaa
aatacggagt ttatgaggcc atattcgcca tgctgtcttc tgtgatgaac aaagacggga
tgctggtagc gtatggaaat ggggtttataa ctctggaatt cctaaaaagc ctaaggaaac
cgctctgtga tatcatggaa cccaagtctg attttgccat gaagtccaat gcactggaac
tggaatgacag tgatatctcc cttttgtcgg ctgctatcat ttgctgtgga gatcgtcctg
gccttctaaa cgtaggacac attgaaaaaa tgcaggaggg tactgtacat gtgctcagac
tcacctgca gagcaaccac ccggacgata tctttctctt cccaaaactt cttcaaaaaa
tggcagacct ccggcagctg gtgacggagc atgcgcagct ggtgcagatc atcaagaaga
cggagtcgga tgctgcgctg caccgcctac tgcaggagat ctacagggac atgtactgag
ttccttcaga tcagccacac cttttccagg agttctgaag ctgacagcac taaaaaggag
acggggggagc agcacgattt tgcacaaata tccaccactt taaccttaga gctcggacag
tctgagctgt aggttaaccgg catattatct catatctttg ttttaaccag tacttctaag
agcatagaac tcaaatgctg ggggagggcg ctaatctcag gactgggaag (SEQ ID NO: 1)

```

FIGURE 2

3/3

MVDTESPLCPLSPLEAGDLESPLSEEFLLQEMGNIQEISQSIGEDSSGSFGFTEYQYLGSCPGSDGSVITDTLSPA
SSPSSVTYPVVPGSVDESPSGALNTECRICGDKASGYHYGVHACEGCKGFFRRTIRLKLVDKCDRSCKIQK
KNRNKCQYCRFK CLSVGMSHNARFGRMPRSEKAKLKAELTCEHDIEDSETADLKSLAKRIYEA YLKNF
NMNKVKARVILSGKASNNPPFVIHDMETLCMAEKT LVAKLVANGIQNKEAEVRIFHCCQCTSVETVTELTE
FAKAIPGFANLDLNDQVTLLKYGVYEAIFAMLSVMNKGMLVAYGNGFITREFLKSRLKPFCDIMEPKFD
FAMKFNALDSDISLFVAAIICCGDRPGLLNVGHIEMQEGIVHVLRLHLQSNHPDDIFLFPKLLQKMAD
LRQLVTEHAQLVQIIKKT ESDAALHPLLQEYRDMY (SEQ ID NO: 2)

FIGURE 3

**This Page is Inserted by IFW Indexing and Scanning
Operations and is not part of the Official Record**

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ BLACK BORDERS
- ☐ IMAGE CUT OFF AT TOP, BOTTOM OR SIDES
- ☐ FADED TEXT OR DRAWING
- ☒ BLURRED OR ILLEGIBLE TEXT OR DRAWING
- ☐ SKEWED/SLANTED IMAGES
- ☐ COLOR OR BLACK AND WHITE PHOTOGRAPHS
- ☐ GRAY SCALE DOCUMENTS
- ☐ LINES OR MARKS ON ORIGINAL DOCUMENT
- ☐ REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY
- ☐ OTHER: _____

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.